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Datasheet

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StepSkip™ Human ADP/Acrp30(Adiponectin) ELISA Kit

Cat# E5012

Storage at 2-8℃ for six months

INTRODUCTION

This ELISA kit applies to the in vitro quantitative determination of Human ADP concentrations in serum, plasma. Please consult technical support for the applicability if other biological fluids need to be tested.

SPECIFICATION

Sensitivity: 0.18ng/mL

Detection Range: 0.39-25ng/mL

Specificity: This kit recognizes Human ADP in samples. No significant cross-reactivity or interference between Human ADP and analogues was observed.

Repeatability: Coefficient of variation is < 10%

PRINCIPLE of KIT

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human ADP. Samples (or Standards) and biotinylated detection antibody specific for Human ADP are added to the micro ELISA plate wells. Human ADP would combine with the specific antibody. Then Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human ADP, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Human ADP. You can calculate the concentration of Human ADP in the samples by comparing the OD of the samples to the standard curve.

CONTENTS and STORAGE

An unopened kit can be stored at 2-8℃ for six months. After test, the unused wells and reagents should be stored according to the table below.

Item	Specifications	Storage conditions after test
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	2-8°C, 1 month
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	Discard unused reconstituted standard and dilutions

Reference Standard & Sample Diluent	1 vial, 20 mL	2-8°C
Biotinylated Detection Ab Diluent	1 vial, 6 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL 24T: 1 vial, 60 µL	2-8°C (Protect from light)
Substrate Reagent	1 vial, 10 mL	
Stop Solution	1 vial, 10 mL	2-8°C
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

OTHER SUPPLIES REQUIRED

- Microplate reader with 450 nm wavelength filter
- High-precision transfer pipette, EP tubes and disposable pipette tips
- Incubator capable of maintaining 37℃
- Deionized or distilled water
- Absorbent paper
- Loading slot for Wash Buffer

NOTE

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should have a 450 (±10 nm) filter installed and a detector that can detect the wavelength. The optical density should be within 0~3.5.
5. Do not mix or use components from other lots.
6. Change pipette tips in between adding standards, in between sample additions, and in between reagent additions. Also, use separate reservoirs for each reagent.

SAMPLE COLLECTION

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 15min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

NOTE for SAMPLE:

1. Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.
2. Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. Bring samples to room temperature and mix gently.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
4. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
5. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
6. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

REAGENT PREPARATION

1. Bring all reagents to room temperature (18~25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water to prepare 750mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 2000pg/mL(or add 1.0mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 2000、1000、500、250、125、62.5、 31.25、0pg/mL.
4. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100µL/well). In

preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent.

ASSAY PROCEDURE

1. Add the Standard working solutionto the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (50 µL for each well). Add the samples to the other wells (50 µL for each well). Immediately add 50µL of Biotinylated Detection Ab working solutionto each well. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃.Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Aspirate or decant the solution from each well，add 350µL of wash bufferto each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washercan be used in this step and other wash steps.
3. Add 100µL of HRP Conjugate working solutionto each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
4. Aspirate or decant the solution from each well, repeat the wash process for 5times as conducted in step 2.
5. Add 90µL of Substrate Reagentto each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
6. Add 50µLof Stop Solutionto each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

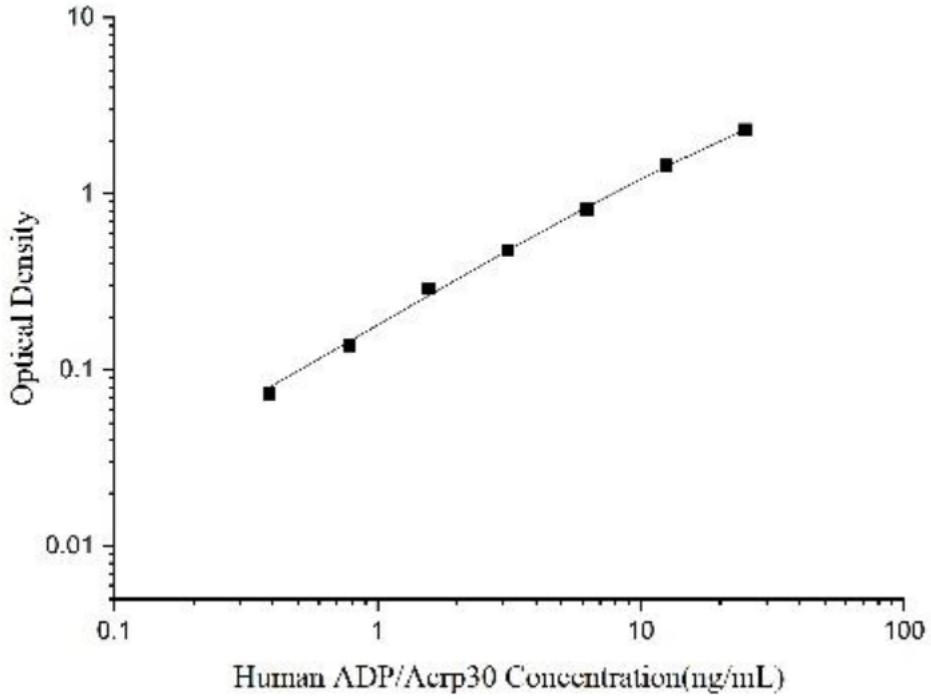
CALCULATION of RESULTS

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

TYPICAL DATA

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipettingtechnique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(pg/mL)	25	12.5	6.25	3.13	1.56	0.78	0.39	0
OD	2.384	1.519	0.888	0.547	0.36	0.208	0.143	0.07
Corrected OD	2.314	1.449	0.818	0.477	0.29	0.138	0.073	-



Sample values

Serum/Plasma/Urine–Samples from apparently healthy volunteers were evaluated for the presence of HumanADPin this assay.

Sample Type	Source	Range	Dilution Factor
Serum（n=12）	Healthy human	7.89-38.2μg/mL	5000-20000
EDTA plasma（n=12）	Healthy human	1.5-28.5μg/mL	5000-20000

PRECISION

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level HumanADPwere tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level HumanADPwere tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	1.09	2.57	12.28	1.24	2.82	11.2
Standard deviation	0.06	0.12	0.49	0.07	0.14	0.55
CV (%)	5.5	4.67	3.99	5.65	4.96	4.91

RECOVERY

The recovery of Human ADP spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	97-109	102
EDTA plasma (n=8)	87-99	92

LINEARITY

Samples were spiked with high concentrations of Human ADP and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

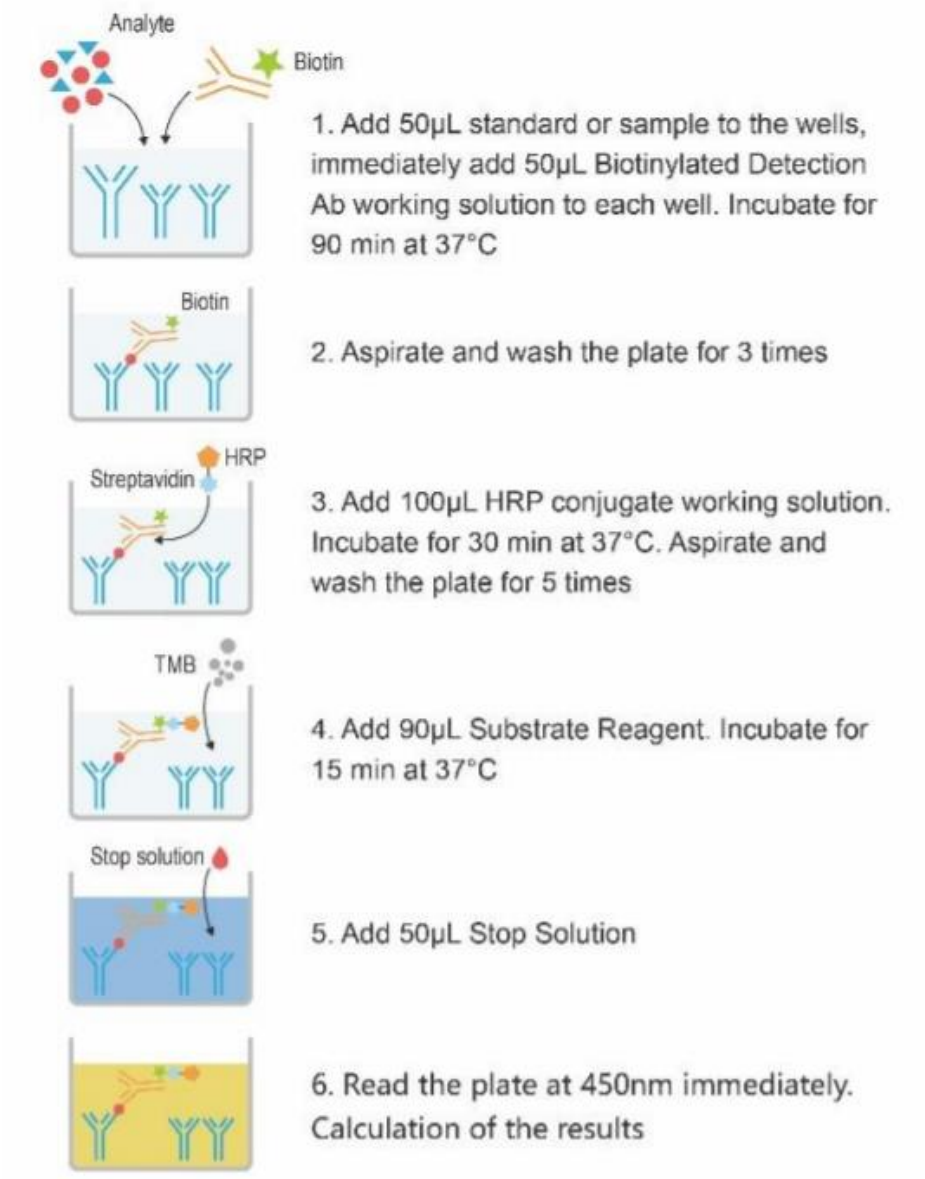
		Serum (n=4)	EDTA plasma (n=4)
1:2	Range (%)	98-108	91-104
	Average (%)	102	96
1:4	Range (%)	90-102	99-108
	Average (%)	95	105
1:8	Range (%)	89-101	96-105
	Average (%)	97	101
1:16	Range (%)	91-102	97-106
	Average (%)	94	102

TROUBLESHOOTING

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting	Check pipettes.

High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

SUMMARY



DECLARATION

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on.We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test.Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.

PRODUCT USE LIMITATION

These products are intended for research use only.